ACCURACY AND PRECESION OF TOTAL NITROGEN DETERMINATION USING AUTOMATED DRY COMBUSTION

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INTRODUCTION

For many years Kjeldahl digestion has been the standard method used for total nitrogen analysis. This technique involves the use of concentrated acids and very strong bases. The method is not only time consuming, but it is also dangerous. While the Dumas method (dry combustion) for total nitrogen analysis has been known for some time, until recently it has not been widely used. With the technological advances of today, the dry combustion technique for total N determination has become more feasible and safer than the Kjeldahl digestion procedure. The dry combustion procedure employs high temperatures in a stream of helium gas and a catalyst to "combust" the sample and release N as N_2 gas. This procedure takes only 3-6 minutes vs the 7-10 hr for Kjeldahl digestion and distillation and uses no acids or bases which makes it faster and less hazardous.

There are numerous instrument manufacturers which have taken advantage of this Dumas technique. The objective of this research was to compare one of these instruments (manufactured by Carlo Erba, Milano Italy) with that of the Kjeldahl procedure.

ACCURACY AND PRECISION OF TOTAL NITROGEN DETERMINATION USING AUTOMATED DRY COMBUSTION ANALYSIS

ABSTRACT

Automated dry combustion procedures have recently replaced more common Kjeldahl digestion methods for total N in soils and plant tissue. Although the precision levels of Kjeldahl and dry combustion total N analysis methods have been reported to be similar, (± 0.01%), comparisons of correlation between these methods has not been extensively evaluated. In addition, techniques to improve the precision associated with total N dry combustion procedures have not been investigated. Soil and plant samples were analyzed for total N using micro-Kjeldahl digestion and dry combustion (Carlo-Erba NA 1500). Dry combustion total N analysis on a wide range of soils was further evaluated using different certified reference standards. Liquid standards (0.1 to 0.0001 %N) were prepared using KNO_3 to determine the detection limits of the dry combustion analyzer. All soils, liquid KNO_3 standards, and certified reference standards

were also analyzed using a modified Kjeldahl digestion procedure. Simple correlation coefficients from linear regression of Kjeldahl N on dry combustion N were 0.88, 0.92 and 0.88 for grain, straw and soil samples, respectively. In general, the Kjeldahl method underestimated dry combustion N in grain, straw, and soil samples. Dry combustion analysis on the 0.0050 %N KNO_3 liquid standard was 0.0048±0.0002 %N. Accurate readings using the Kjeldahl digestion procedure could only be obtained on samples with > 0.01 %N KNO_3. Significant differences in slopes were obtained when different reference standards were used. The lowest reference standard used (Tibetan soil, 0.128 %N) significantly narrowed the range of % N determined on a random population of local soils. Present recommendations from the instrument manufacturer which suggest that the use of isothioruea (8.13 %N) as a single reference standard were not applicable to this work since slopes were altered. The use of the Tibetan soil as a calibration standard improved the precision of dry combustion total soil N on samples with less than 0.15%N.

LITERATURE REVIEW

Evaluations of dry combustion methods for total N and organic C analysis have concentrated on plant tissue analysis while relatively little has been done on soils (Artiola, 1989; Kersten and Jansson, 1986; and Jensen, 1991). The precision of these instruments is reported to be equal to that of Kjeldahl digestion (Carlo Erba, 1990; Artiola, 1989). The Carlo Erba NA 1500 (Carlo Erba, Milano, Italy) advertises its precision to be ±0.01%. Due to an extremely small sample size (approximately 15 mg), heterogeneous materials must be finely ground when using dry combustion procedures (Kersten and Jansson, 1986; and Jensen, 1991).

Wet digestion procedures for analyzing total N require the use of strong acids and catalysts (Se and/or Hg) which generate a significant amount of waste from (Artiola, 1989). Jones (1987) noted the importance of identifying which specific procedures were employed for total N determination using Kjeldahl digestion since various modifications are presently employed depending on whether nitrate is included in the determination. Work by Schuman et al. (1973) evaluated the use of a Tecator digestion block and Technicon AutoAnalyzer as a reliable replacement for micro-Kjeldahl procedures. This method eliminated the distillation and titration steps used in Kjeldahl procedures and increased the number of samples which could be analyzed. The use of

ammonium electrode semiautomated procedures have been discussed by Gallaher et al. (1976). Studies by Hamlyn and Gasser (1970) found improved micro-Kjeldahl N results when grinding plant materials to pass 0.25 mm screens compared to 1.0 mm. Nelson and Sommers (1973) developed a modified procedure for estimating total N in plant tissue containing high nitrate concentrations. Various authors have established the correlation between modified total N methods and Kjeldahl digestion techniques for plant and soil samples (Vincent and Shipe, 1976,).

Recent studies by Morra et al. (1991) found that the use of near-infrared reflectance spectroscopy can be used to predict C and N concentrations in soil size fractions. Mulvaney et al. (1992) demonstrated that K_2SO_4 could be used in place of KCl for extraction of inorganic N from soil in order to avoid potential Cl^- interference.

It has been reported that lower results are generally obtained using the wet digestion technique when compared to dry combustion methods (Starr et.al 1984). This may be a result of wet digestion methods failing to recover N in heterocyclic compounds and/or N in N-N or N-O linkages (Jensen, 1991). Although various authors have compared total N results using different methods (Artiola, 1989; Tabatabai and Bremner, 1991), the precision and accuracy of dry combustion techniques have not been thoroughly evaluated. The objectives of this research were to evaluate

the methodology available for determining total N (by dry combustion) in soil and plant tissue and to establish the relationship between total Kjeldahl N analysis and dry combustion N analysis on soil and plant tissue samples.

MATERIALS AND METHODS

The Carlo Erba dry combustion analyzer was set up according to manufactures instructions. The instrument combusts the sample at approximately 1700 °C in a stream of helium which is used as a carrier. Sample gases then flow through hot chromium oxide (1020 °C) and silvered cobalt to remove $SO4^{=}$ and halogens. The sample then passes through hot copper to reduce any oxides of nitrogen to N_2. After passing through $MgHClO_4$ to remove water, the sample enters a Poropaq[a] chromatographic column where N, C and S can be separated into individual peaks which are read using a thermal conductivity detector (Carlo Erba, 1990). Presently, the use of only one standard is recommended for determinating the linear calibration curve (Carlo Erba, 1990).

A number of experiments were conducted to evaluate the stated objectives.

Direct Method Comparison:

Surface soil (0-15cm), wheat straw, and wheat grain

samples were analyzed for total N by a modified micro-Kjeldahl digestion which includes NO_3(Bremner, 1960) using a Tecator digestion block and by the dry combustion procedure (Carlo-Erba NA 1500). A total of 180 surface soils (0-15 cm) from seven locations in Oklahoma were included in this analysis in order to obtain a wide range in total soil N. Similarly 120 plant samples were analyzed with a wide total N range. Grain samples were placed in a drying oven at 75° C for 2 hours in order to remove excess moisture.

For the total Kjeldahl N (TKN) analysis, soil samples were weighed (0.50g) into digestion flasks where they were pretreated with $KMnO_4$ and reduced iron to convert NO_3^- and NO_2^- to NH_4^+ prior to digestion. A catalyst of K_2SO_4, $CuSO_4$, and Se (100:10:1) was used for both soil and plant digestion. Nitrogen in the digest was then determined using a 'Lachat QuickChem' automated flow injection analysis system. Linear regression of Kjeldahl N versus dry combustion N was performed and linear regression on duplicate samples, by procedure was also evaluated. Analysis of variance was performed on grain, straw, and soil samples using procedures as main experimental units.

Recovery of Nitrogen from Pure Solutions:

Standard solutions containing 0.1 to 0.0001% N were analyzed using the Carlo Erba NA 1500 C/N/S dry combustion autoanalyzer and Kjeldahl digestion for total N. Standard

solutions were prepared using KNO_3 as the N source. A 1% stock solution was prepared by weighing 72.143g of KNO_3 and bringing to a volume of one liter with deionized water. Further dilution of 100, 50, 10 and 5 ml of the stock solution to one liter resulted in solutions of 0.1, 0.05, 0.01, and 0.005% N. In a similar manner the 0.1% solution was used to make 0.001, 0.0005, and 0.0001% N standard solutions using 10, 5, and 1 ml, respectively.

For the dry combustion total N analysis, chromosorb W was added to each of the sample capsules which were to contain the standard solutions. Chromosorb W is an inert compound used to absorb liquid in the capsule. The capsule containing the Chromosorb W was then placed on the balance and tared. Approximately 50 mg of standard sample was placed (using a bulb pipet) in the capsule and immediately weighed. Each standard solution was analyzed as a sample five times in sequential order. Once weighed, samples were dried in a forced air oven at 75 °C for 2 hrs. Capsules were then sealed and placed in the sample carousel. This procedure was repeated once for each of three certified standards (standards certified by National Institute of Standards and Technology). Dry combustion total N was determined on the KNO_3 standard solutions on three separate occasions using; a) corn stalks (0.697±0.032 %N), b) phenanthrene (0.178± %N) and c) Tibetan soil (0.128±0.005 %N) as the certified reference materials for standardizing

the instrument. Kjeldahl total N analysis on the liquid N standards was determined as described in experiment #1. Dry combustion and total Kjeldahl N analysis of all the standards (prepared liquid standards and certified reference standard) were compared to the certified or calculated value for either dry combustion or Kjeldahl digestion methods. For this experiment Tibetan soil was used to calibrate the dry combustion instrument. Linear regression of the actual N concentration and that observed from dry combustion and Kjeldahl analysis was performed.

Certified Standard Comparison:

At present, only one certified standard (5-Chloro-4-hydroxy-3-methoxy-benzylisothiourea phosphate, also known as isothiourea, containing theoretically 8.13 %N) is recommended for use in establishing the slope and intercept for independent sample analysis. In order to determine the accuracy of the slope, certified N standards (corn stalks, phenanthrene, and a Tibetan soil containing 0.697, 0.178 and 0.128 %N, respectively) were independently used as the reference standard for total N analysis. Three separate slopes were determined using each independent certified standard. When one standard was used as the calibrating material, the other two were run as reference samples.

Chemical Standard Comparison:

Twenty randomly selected soils were analyzed in duplicate for total N using either isothiourea (8.13% N) or a 0.2 %N NH_3NO_3 prepared solution standard as the instrument calibration standards. A 1% stock solution was prepared by weighing 28.586g of NH_4NO_3 and bringing it to a volume of one liter with deionized water. 200 ml of this stock solution was then diluted to 1 liter to give a 0.2% N solution. The isothiourea was obtained from Fisons instruments Danvers Ms. The samples were analyzed on two separate occasions one day apart. A 0.01% N prepared solution standard was also analyzed (in 5 replicates) for each calibration standard each day. Analysis of variance was performed on both the 0.01% solution standard and the soils using standards as main experimental units split by time.

For each of these analysis, except for use of the three different calibration standards, the dry combustion instrument was setup as prescribed by the manufacturers specification for total N.

RESULTS AND DISCUSSION

Direct Method Comparison:

Simple correlation coefficients from linear regression of Kjeldahl N versus dry combustion N were 0.94, 0.95 and 0.84 for grain, straw and soil samples, respectively (Figure

1). The standard used for calibrating the NA-1500 dry combustion analyzer was Tibetan soil (0.128±0.005 %N) and apple leaves (2.25±0.19 %N, NIST) for soil and plant samples, respectively. As is illustrated in Figure 1, slope components were altered depending on the type of sample analyzed and/or the material used to standardize the dry combustion instrument. Total N analysis was higher on grain samples when using dry combustion while only small differences between procedures were found for straw samples. Soil N analysis revealed a different relationship. When TKN values were low (< 0.02% N), DCN values were 2x higher. Given the large intercept component and lower slope, this suggests that the TKN detection limit had been exceeded in the lower range. At higher sample N concentrations (0.10% N) the methods were equal.

Linear regression was also performed on the duplicate sample analysis of each procedure (TKN-1 vs. TKN-2 and DCN-1 vs DCN-2) to evaluate procedural errors or precision (Figures 2 and 3 and Table 1). Duplicate analysis of soil samples showed less precision using TKN than DCN as illustrated by the increased scattering for TKN duplicates and decreased correlation (Figures 2 and 3). However, there was a tendency for the opposite to be true on grain N analyses. This could suggest that procedural errors from the TKN analyses (such as weight difference of catalyst or iron added to digest, contamination of reagents, inaccurate

weights, ect.)were greater than when using dry combustion. Analysis of variance on total N analysis for soil, grain and straw samples assigning methods as main experimental units is reported in Table 2. For all three types of samples analyzed, there were significant differences in methods indicating that the overall means were altered depending on how N was analyzed. For all three types of samples analyzed, N values were greater when using dry combustion compared to micro-Kjeldahl digestion (Table 2).

Recovery of Nitrogen from Pure Solutions:

The principle reason for preparing liquid standards at concentrations less than the advertised precision of the Carlo Erba NA 1500 dry combustion analyzer was to determine if the detection limits were lower than 0.01%N. Using three separate certified standards as reference materials, the detection limit for %N was 0.005 as no readings were obtained on the 0.001 %N standards (Table 3). The precision levels using the 0.0050 %N standard averaged ±0.0002 (Table 3). Using the three separate reference standards, percent recovery at the 0.005% N level was 89.2%, 51.0% and 84.6%, for corn stalks, phenanthrene and Tibetan soil, respectively. Therefore, while precision is excellent, accuracy is very poor. Nitrogen was not detected at %N levels less than 0.005 (0.001, 0.0005 and 0.0001). However, it is important to note that obtaining precision levels of

±0.0002 for the 0.005 %N standard indicates that total N concentrations in solutions can be determined at concentrations as low as 50 μg/g (0.005% x 10,000). Both Kjeldahl and dry combustion procedures have been reported to have ±0.01%N or ±100 μg/g precision levels. Consequently because of this, it has been impossible to detect differences in total soil N in two or three year experiments evaluating rates of applied N. If a range of N rates were selected in a field experiment where the differences between rates were more than 10 μg/g (e.g. > 0.001 precision level), estimates of differences in total soil N as a dependent variable could conceivably be obtained.

When solution KNO_3 standards were analyzed using Kjeldahl digestion, no N could be detected when standards were less than 0.01 %N (Table 3). This is consistent with other findings on the detection limits of the Kjeldahl procedure and associated standard deviations (Table 4). However, it is important to note that an NH_4-N source of N would have been preferable in this experiment thus avoiding potential errors associated with incomplete reduction (using devarda's alloy) of the NO_3-N source (KNO_3) employed.

Slopes from regression of percent N observed on percent N prepared were significantly different (test not shown) from one another when the reference standard was changed (Figure 4). In each of these regression equations, the KNO_3 standard solution was analyzed five times and, three

separate certified reference standards were used for calibrating the linear relationship between prepared and observed N. Intercepts were not different from zero for any of the calibration curves, however, differences in slope components suggest that regression linearity was altered depending on which standard was used. Using corn stalks as the reference standard, slopes were not significantly different from 1.0 (both A and B, Figure 4). However, when phenanthrene and Tibetan soil were used as the reference standards, slopes were significantly less than 1.0. This suggests that percent N was underestimated when the reference standards contained lower levels of N. These results are consistent with that reported in Table 3 where the 0.1 %N KNO_3 standard had significantly lower observed values as the %N in the reference standard decreased.

Certified Standard Comparison:

When the certified standards (corn stalks, phenanthrene, Tibetan soil) were analyzed as samples,alternately using one of them as the standard for calibration, similar results were obtained to that of Experiment #2 (Figure 5). Total N was overestimated for corn stalks (highest %N of the standards) when either phenanthrene or Tibetan soil were used as the calibration standards (slopes > 1). However, when corn stalks were used as the calibration standard, analyzed values for

phenanthrene and Tibetan soil were both accurate.

Chemical Standard Comparison:

Analysis of variance on the total N analysis for the 0.1% standard (of all replicates) and for the soils data are reported in table 5 and 6. For all analysis there were significant differences in data obtained using different standards to calibrate the instrument. This indicates that the analysis changed depending on the standard used. There was no difference in the days which indicates that the instrument was stable over time. As expected there was a sample by standard interaction. In both experiments the isothiourea (8.13% N) gave lower results than the prepared solution standard (0.2% N). The variability in the results obtained from the different calibration standards could possibly be due to the instrument not being totally linear throughout the range which was analyzed. The variability is more likely to be due to the instrumental error inherent in the instrument. This is to say that while the error is the same at both ends of the scale (8.13% and 0.2%) the differences associated with it are magnified at the low end. This also indicates that what has previously been recommended by instrument representatives (use of benzylisothiourea 8.13% N as a common standard) is not accurate when analyzing samples with low total N.

CONCLUSIONS

In general, dry combustion N analysis was more precise than total Kjeldahl N when duplicate samples were regressed on one another. Total N in grain, straw, and soil samples was higher when N was determined using dry combustion compared to micro-Kjeldahl. Precision levels on readings from prepared liquid KNO_3 standards were apparently better than the ±0.01%N advertised (detection to 0.005 ±0.002). This work suggests that the %N determined using dry combustion can be accurately detected at 0.005% N levels. Significant differences in slopes were found when different reference standards were used suggesting that the accuracy or bias in sample determinations was altered by the standard employed. The lowest %N reference standard used (Tibetan soil, 0.128 %N) significantly narrowed the range of values read from a random population of local soils which would indicate that analytical precision is altered by the standard employed. Because only one standard is recommended for establishing the calibration curve, this study suggests using a %N reference standard just slightly above the expected range in samples to be analyzed.

References

Artiola, Janic F. 1989. Determination of carbon, nitrogen and sulfur in soils, sediments and wastes: A comparative study. Intern.J. Environ. Anal. Chem. 41:159-171.

Bremner J.M. 1960. Determination of nitrogen in soil by the Kjeldahl method. J. Agric. Sci. 55:11-33.

Carlo Erba Instruments. 1990. Nitrogen Analyzer 1500 Series 2 Instruction Manual. Milan, Italy.

Kersten, W.G., and Kjell H. Jansson. 1986. Rapid and automatic determination of nitrogen using flash combustion of large samples. Anal. Chem. 58:2109-2112.

Gallaher, R.N., C.O. Weldon, and F.C. Boswell. 1976. A semiautomated procedure for total nitrogen in plant and soil samples. Soil Sci. Soc. Am. J. 40:887-889.

Hamlyn, F.G., and J.K.R. Gasser. 1970. Some causes of error in determination of total nitrogen in plant material. Chemistry and Ind. August, 1142-1143.

Isaac, Robert A., and William C. Johnson. 1976. Determination of total nitrogen in plant tissue using a block digester. J. Assoc. Of. Anal. Chem. 59:98-100.

Jensen, E.S. 1991. Evaluation of automated analysis of ^{15}N and total N in plant material and soil. Plant and Soil 133:083-92.

Jones, J. Benton Jr. 1987. Kjeldahl nitrogen determination - what's in a name. J. Plant Nutr. 10:1675-1682.

Tabatabai, M.A., and J.M. Bremner. 1991. Automated instruments for determination of total carbon, nitrogen, and sulfur in soils by combustion techniques. In K.A. Smith (ed.) Soil analysis, modern instrumental techniques. Second Edition. Marcel Dekker, inc., New York, N.Y.

Morra, M.J., M.H. Hall, and L.L. Freeborn. 1991. Carbon and nitrogen analysis of soil fractions using near-infrared reflectance spectroscopy. Soil Sci. Soc. Am. J. 55:288-291.

Morris, G.F., R.B. Carson, D.A. Shearer, and W.T. Jopkiewicz. 1968. Comparison of the automatic dumas (Coleman model 29A nitrogen analyzer II) and Kjeldahl methods for the determination of total nitrogen in agricultural minerals. J. Assoc. Of. Anal. Chem. 51:216-219.

Mulvaney, R.L., F. Azam, S.N. Stein, and F.W. Simmons. 1992.

Chloride interference in total nitrogen analysis by the Kjeldahl method. Commun. Soil Sci. Plant Anal., 23:1805-1813.

Nelson, D.W., and L.E. Sommers. 1973. Determination of total nitrogen in plant material. Agron. J. 65:109-112.

Schuman, G.E., M.A. Stanley, and D. Knudsen. 1973. Automated total nitrogen analysis of soil and plant samples. Soil Sci. Soc Amer. Proc. 37:480-481.

Starr, C, J. Young, and D.B. Smith. 1984. Measurement of the nitrogen content of plant breeding material using a Carlo Erba nitrogen analyzer. Plant Breeding Institute, Trumpington, Cambridge, U.K.

Vincent, K.R., and W.F. Shipe. 1976. Effect of calibration procedures on accuracy and precision of automated Kjeldahl nitrogen analysis in some formulated foods. J. Food Sci. 41:157-162.

Table 1. Linear regression equations of duplicate soil, grain, and straw samples analyzed for total N using micro-Kjeldahl and dry combustion techniques.

Variables	Intercept	Std. Err. of Estimate	Slope	Std. Err. of Estimate	r	Root MSE
			SOIL			
TKNA VS TKNB	0.006197	0.0195	0.913	0.034	0.91	0.0099
NDCA VS NDCB	0.002784	0.0020	0.963	0.027	0.94	0.0061
			GRAIN			
TKNA VS TKNB	0.500959	0.1038	0.783	0.048	0.84	0.2040
NDCA VS NDCB	0.349392	0.1298	0.875	0.050	0.86	0.2578
			STRAW			
TKNA VS TKNB	0.007168	0.0120	0.995	0.014	0.99	0.0355
NDCA VS NDCB	0.005146	0.0115	0.993	0.012	0.99	0.0316

r - simple correlation coefficient

Table 2. Split plot analysis of variance on soil, grain, and straw samples for total N using procedures as main plots.

Source	df	soil	df	grain	df	straw
		Mean Squares				
SAMP	149	0.001606^{NT}	58	0.721151^{NT}	56	0.215383^{NT}
PROC	1	0.057407**	1	9.946427**	1	0.504449**
SAMP*PROC$^{(a)}$	149	0.000139	58	0.037605	56	0.004876
DUP	1	0.000133*	1	0.010337	1	0.000039
PROC*DUP	1	0.000093$^{@}$	1	0.003091	1	0.000262
SAMP*DUP	149	0.000043**	54	0.055262**	56	0.000638
ERROR	149	0.000002	54	0.005844	56	0.000495
CV,%		8		3		2
SED		0.0117		0.1939		0.0698

	%N		%N		%N	
Procedure	mean	SD	mean	SD	mean	SD
TKN	0.0524	0.0238	2.146	0.401	0.797	0.229
DCN	0.0719	0.0183	2.563	0.508	0.891	0.238

**,*,@ - significant at the 0.01, 0.05, and 0.1 probability levels, respectively.
NT - no statistical test.
SAMP - sample
PROC - procedure
DUP - duplicate
(a) - error a
SD - standard deviation
SED - standard error of the difference between two equally replicated means. (samp*proc used as the error term)

Table 3. Dry combustion N analysis (employing 3 different reference standards) and total Kjeldahl N on liquid KNO_3 standards.

	% N Prepared	NA 1500 % N
DRY COMBUSTION		
Calibration Standard = Corn Stalks (0.689±0.032 %N)		
	0.1	0.1004±0.0034
	0.05	0.0489±0.0034
	0.01	0.0094±0.0001
	0.005	0.0045±0.0002
	0.001	ND
	0.0005	ND
	0.0001	ND
Calibration Standard = Phenanthrene (0.178 %N)		
	0.1	0.0872±0.0019
	0.05	0.0424±0.0008
	0.01	0.0072±0.0003
	0.005	0.0026±0.0002
	0.001	ND
	0.0005	ND
	0.0001	ND
Calibration Standard = Tibetan Soil (0.128±0.005 %N)		
	0.1	0.0694±0.0087
	0.05	0.0405±0.0029
	0.01	0.0090±0.0006
	0.005	0.0048±0.0004
	0.001	ND
	0.0005	ND
	0.0001	ND
TOTAL KJELDAHL		
	0.1	0.096±0.0009
	0.05	0.048±0.0029
	0.01	0.008±0.0013
	0.005	ND
	0.001	ND
	0.0005	ND
	0.0001	ND

ND - not detectable

Table 4. Total N analyses and reported standard deviations on soil, plant tissue and grain samples using different procedures.

Material	Method	%N	SD	Source
Silt loam soil	mK	0.161	0.003	Schuman et al. (1973)
Silt loam soil	TAA	0.177	0.014	Schuman et al. (1973)
Corn grain	mK	1.764	0.031	Schuman et al. (1973)
Corn grain	TAA	1.708	0.017	Schuman et al. (1973)
Corn grain	mK	1.49	0.04	Gallaher et al. (1976)
clay loam soil	mK	0.052	0.0043	Gallaher et al. (1976)
sandy loam soil	mK	0.064	0.0042	Gallaher et al. (1976)
Corn tissue	BD-40	2.68	0.055	Isaac and Johnson (1976)
Wheat grain	mK	2.94	0.03	Morris et al. (1968)

mK - micro-Kjeldahl
TAA - Technicon AutoAnalyzer
BD-40 - Technicon BD-40 digester

Table 5. Split plot analysis of variance on standards and soil samples using standards as main experimental units.

Source standard	DF	0.01 % N Mean Square
DUP	4	0.00000117
DAY	1	0.00000048
STD	1	0.00029489**
DAY*STD	1	0.00000053
CV, %	6	
SED	0.0232	

Standard	%N mean	sd
Isothiourea	0.01717	0.0007
0.2% NH_4NO_3	0.02485	0.0015

% N soil		
REP	1	0.00004471
DAY	1	0.00019014
DUP*DAY	1	0.00000998
SAMP	19	0.00073784**
STD	1	0.00056014**
SAMP*STD	19	0.00023903**
DAY*SAMP	19	0.00004254
DAY*STD	1	0.00018377
DAY*SAMP*STD	19	0.00004720
CV, %	9	
SED	0.0946	

** - significant at the 0.01 probability level
SAMP - sample
DUP - duplicate
SED - standard error of the difference

Table 6. Sample means and standard deviations of soil total N when isothiourea and 0.2% NH_4NO_3 standards were used.

Sample		%N Mean	Standard Deviation
Standard means			
I		0.08863071	0.00994040
S		0.09237285	0.01556850
Sample Means			
1		0.08359062	0.01016587
2		0.08550125	0.00969432
3		0.08815650	0.00661238
4		0.09218100	0.00469130
5		0.08581263	0.00859404
6		0.08637250	0.00514150
7		0.08764887	0.00494557
8		0.10175075	0.01299167
9		0.10698975	0.01483949
10		0.08732300	0.00541982
11		0.09311013	0.00902028
12		0.09081275	0.00836087
13		0.08440300	0.00808331
14		0.09070475	0.00349164
15		0.10852012	0.01854987
16		0.09905437	0.01521086
17		0.09181475	0.00711315
18		0.06426125	0.00279025
19		0.09744963	0.01389369
20		0.08457800	0.00812589
Sample X Standard Means			
1	I	0.08438525	0.00484170
	S	0.08279600	0.01469736
2	I	0.09264475	0.00051045
	S	0.07835775	0.00910751
3	I	0.08568650	0.00552406
	S	0.09062650	0.00743218
4	I	0.09507950	0.00238367
	S	0.08928250	0.00482363
5	I	0.09315575	0.00082734
	S	0.07846950	0.00527817
6	I	0.08470125	0.00349699
	S	0.08804375	0.00648109
7	I	0.08659550	0.00504686
	S	0.08870225	0.00535169
8	I	0.09679000	0.00495969
	S	0.10671150	0.01742427
9	I	0.09521350	0.00325433

Table 6. Con't

	S	0.11876600	0.01155075
10	I	0.08641025	0.00643083
	S	0.08823575	0.00499630
11	I	0.08877050	0.00416940
	S	0.09744975	0.01105664
12	I	0.08429950	0.00244291
	S	0.09732600	0.00663441
13	I	0.09042025	0.00611344
	S	0.07838575	0.00430497
14	I	0.08908275	0.00443927
	S	0.09232675	0.00131303
15	I	0.10818850	0.01817018
	S	0.10885175	0.02173582
16	I	0.08597950	0.00303360
	S	0.11212925	0.00864801
17	I	0.09165825	0.00620024
	S	0.09197125	0.00891914
18	I	0.06379600	0.00288028
	S	0.06472650	0.00304843
19	I	0.09010325	0.01117185
	S	0.10479600	0.01347910
20	I	0.07965350	0.00478967
	S	0.08950250	0.00815235

I - Isothiourea
S - 0.2% NH_4NO_3 solution standard

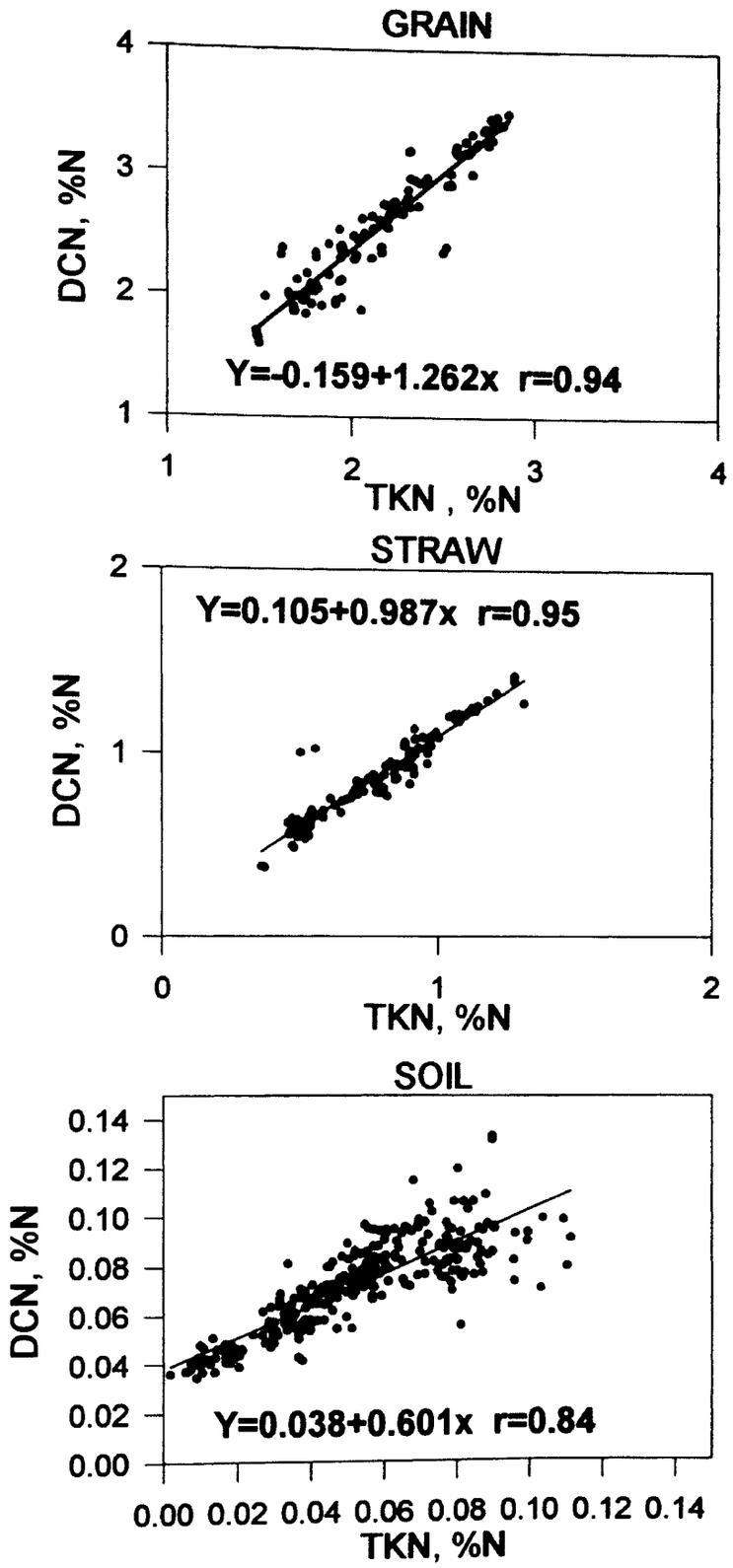


Figure 1. Linear regression equations and plots of total Kjeldahl N (TKN) on dry combustion N (DCN) for grain, straw and soil samples

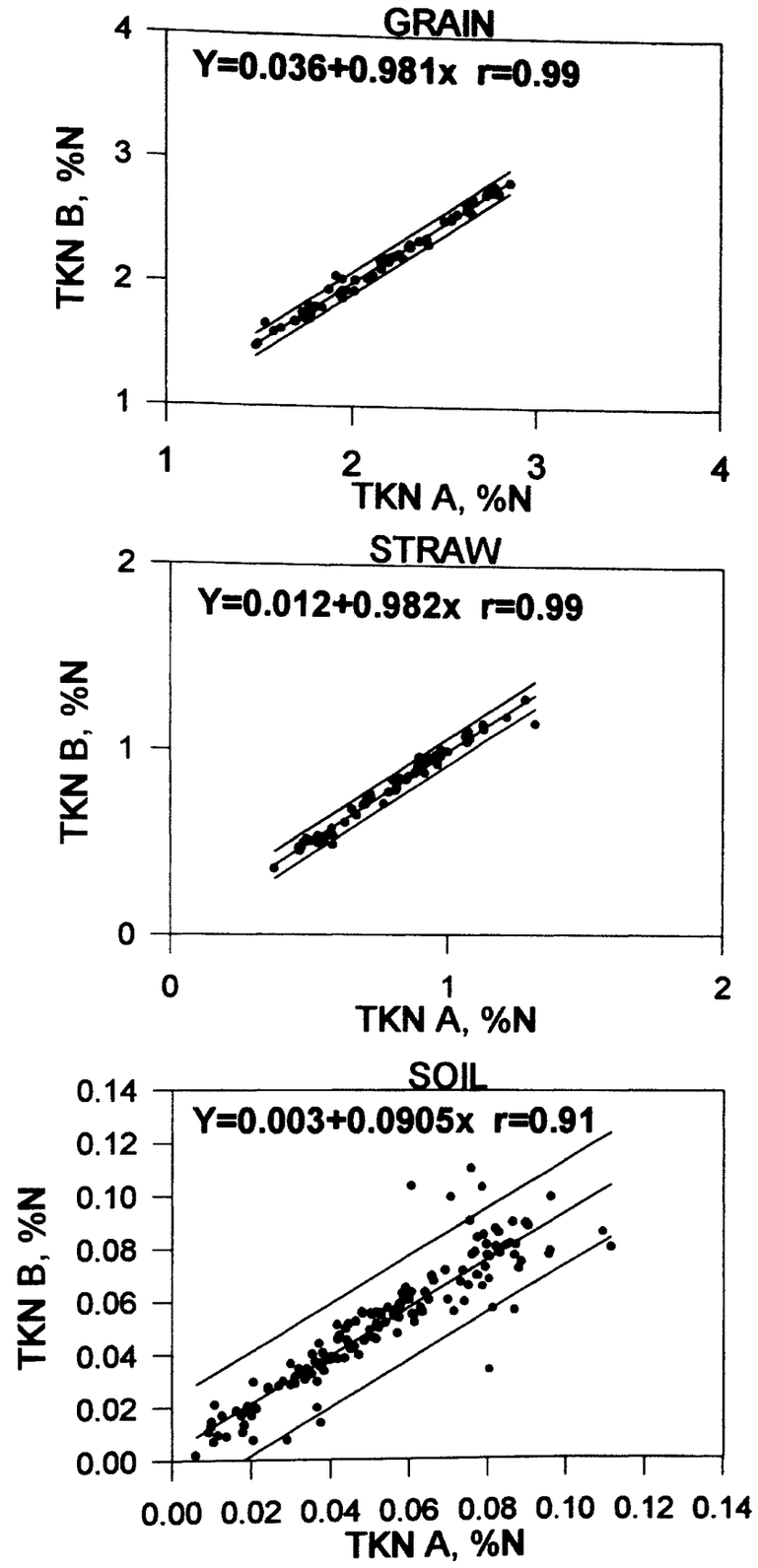


Figure 2. Linear regression equations, 95% confidance limits and plots of duplicate sample analysis (A vs B) for Kjeldahl N determinations on grain, straw, and soil samples.

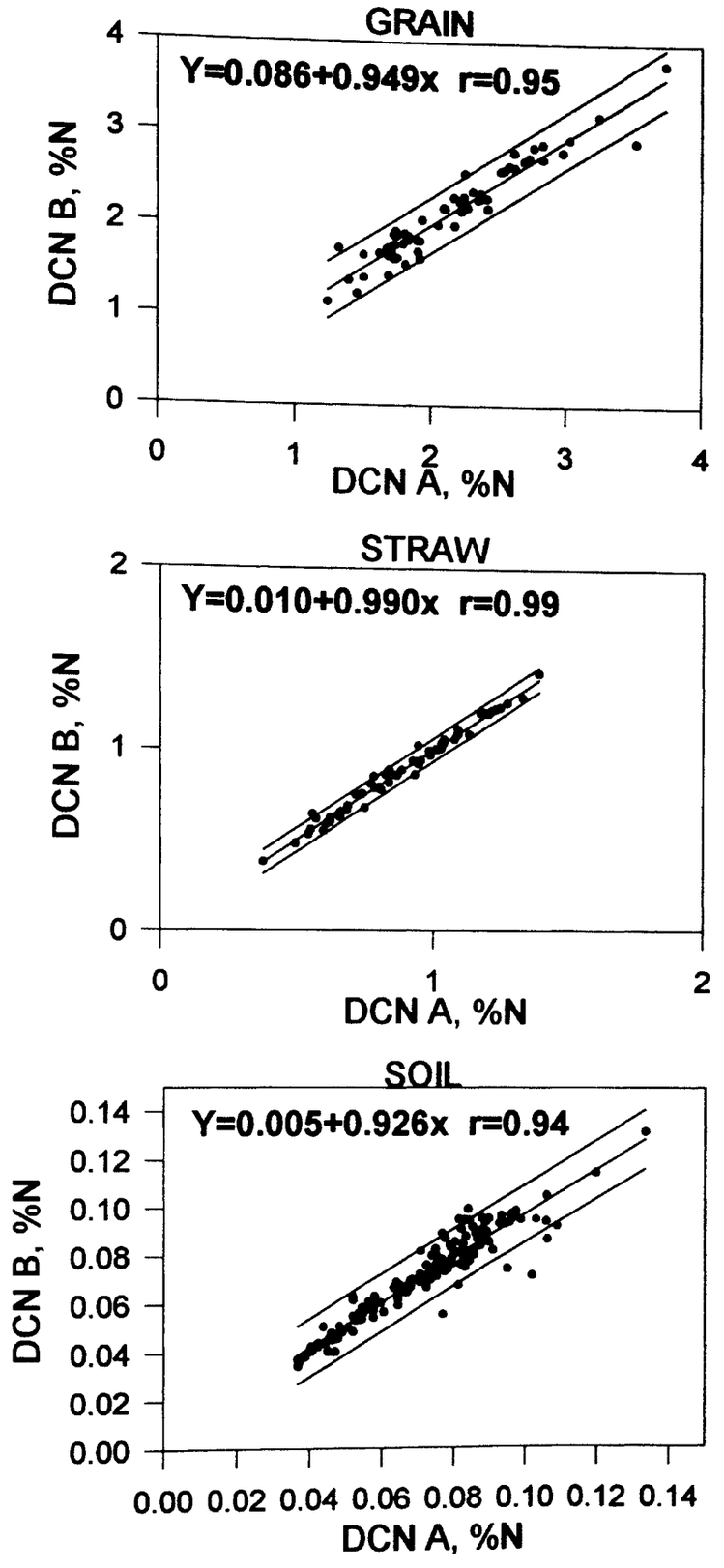


Figure 3. Linear regression equations, 95% confidence limits and plots of duplicate sample analysis (A and B) for dry combustion N determinations on grain, straw and soil samples.

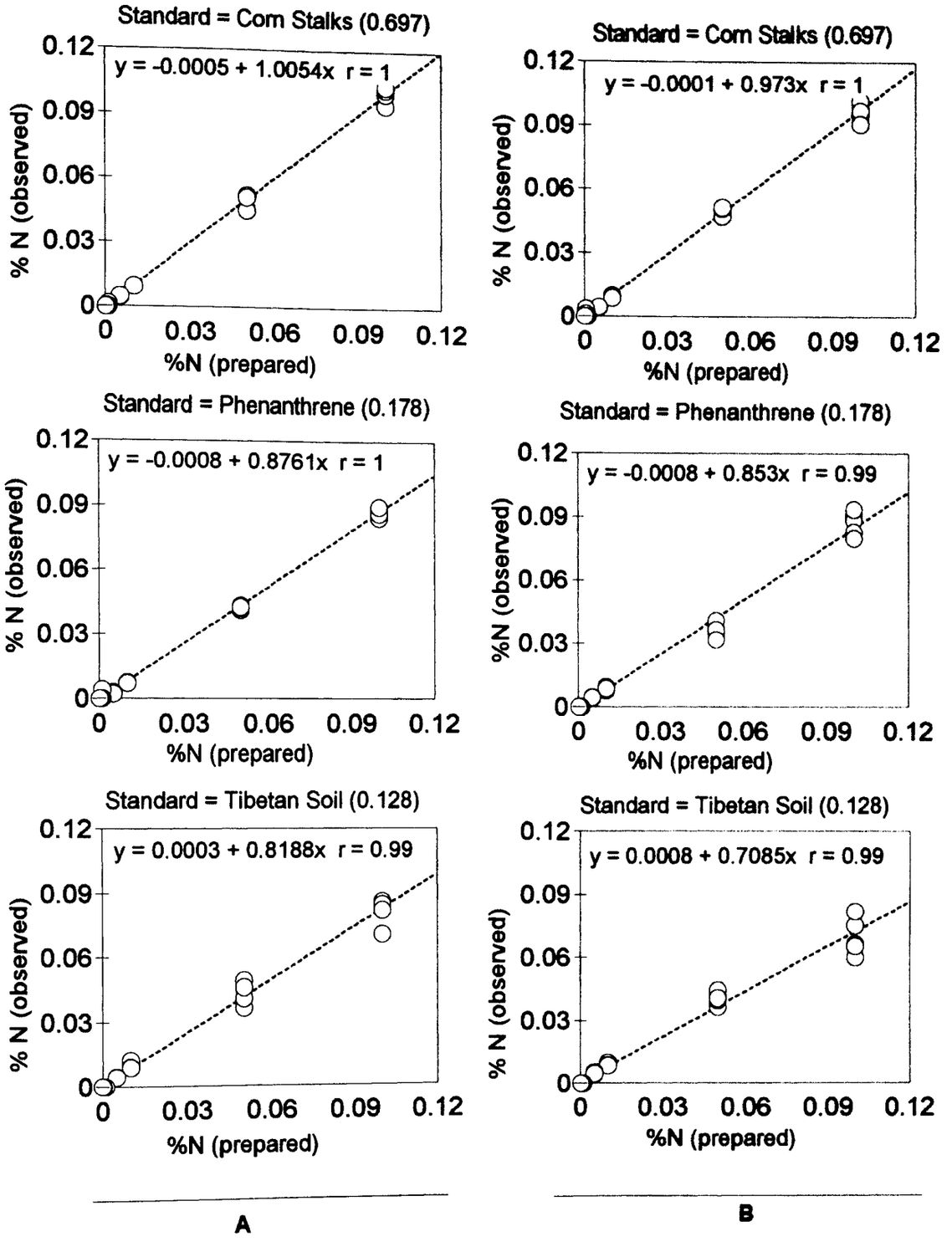


Figure 4. Linear regression of percent N (liquid KNO3 standards) prepared on percent N observed using three certified reference N standards (duplicates A and B).

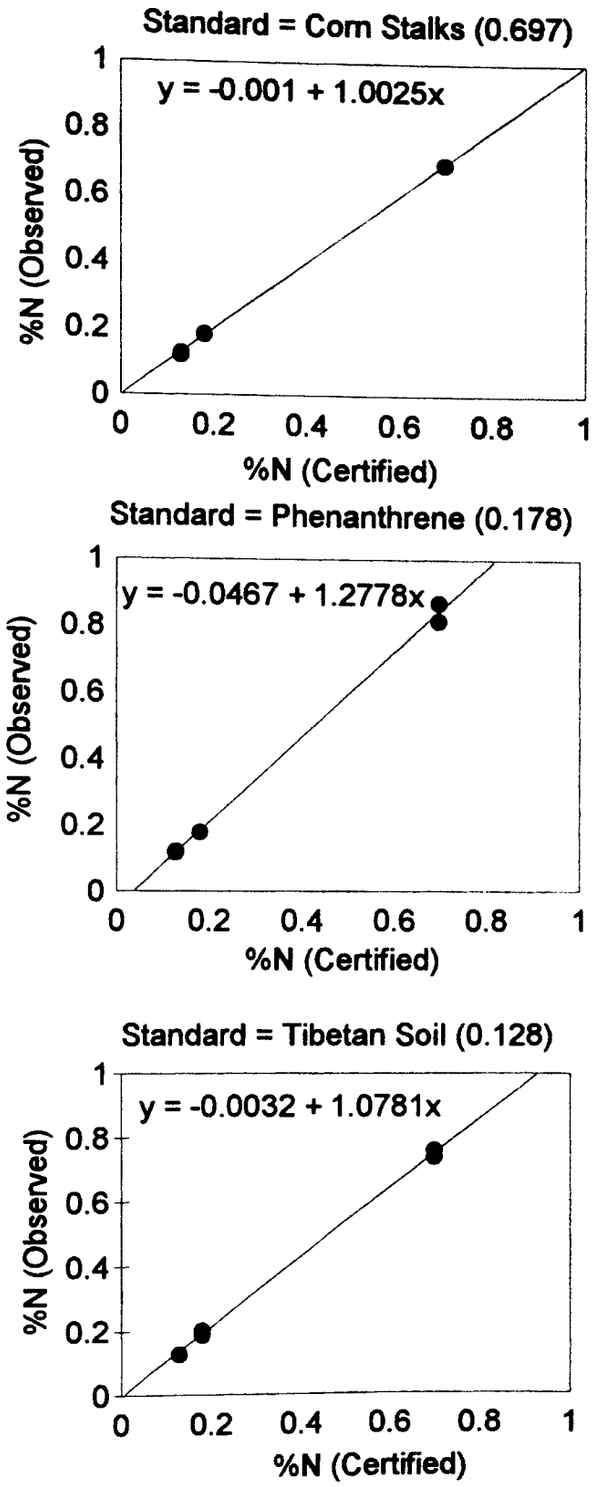


Figure 5. Linear regression of percent N certified on percent N observed using three separate standards as the calibration reference point.

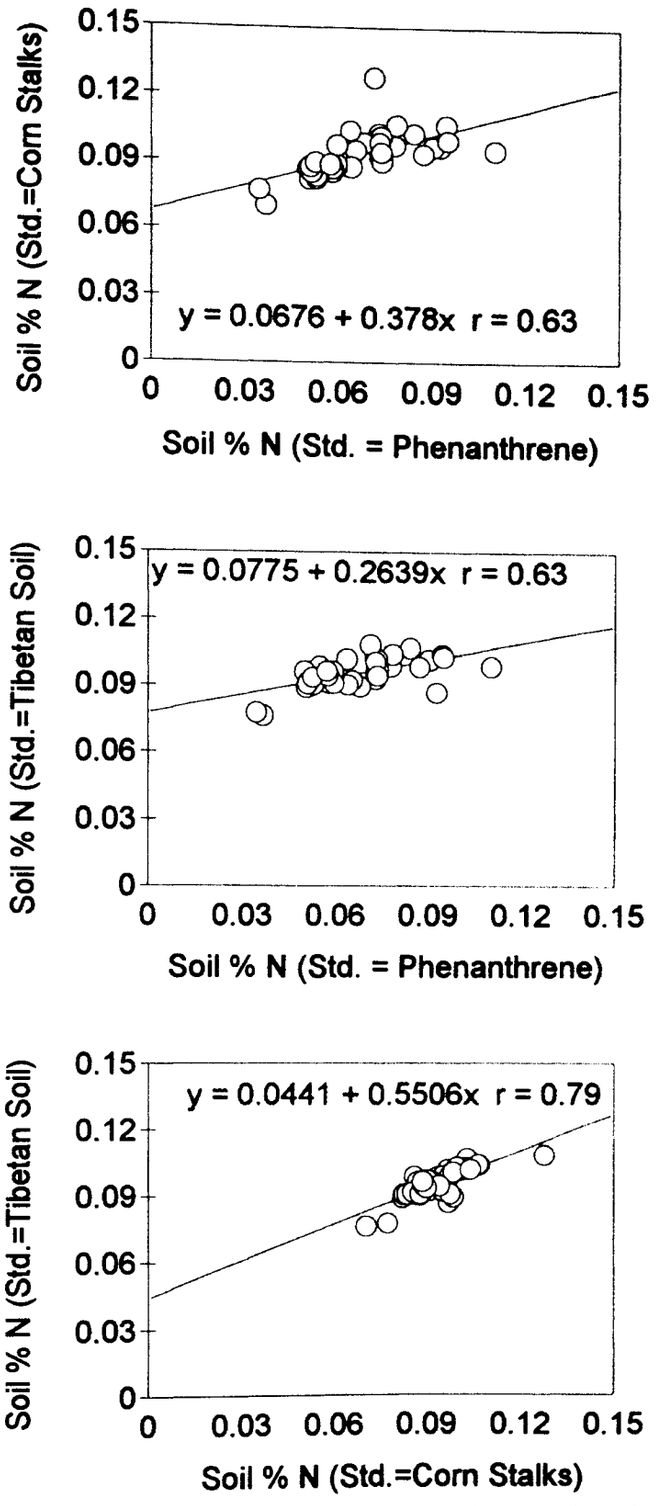


Figure 6. Linear regression of soil N determined using three separate certified standards.

APPENDICES

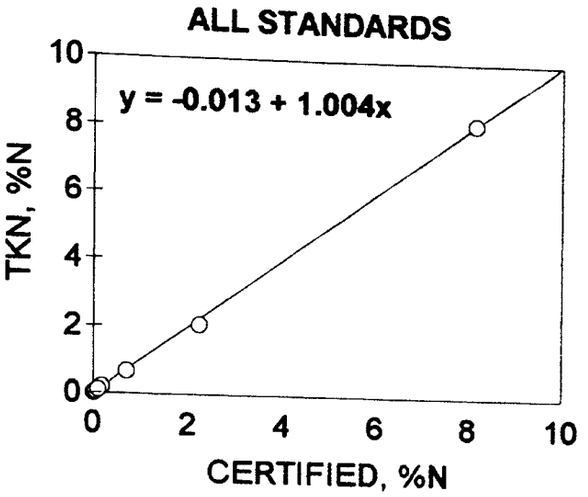
APPENDICES

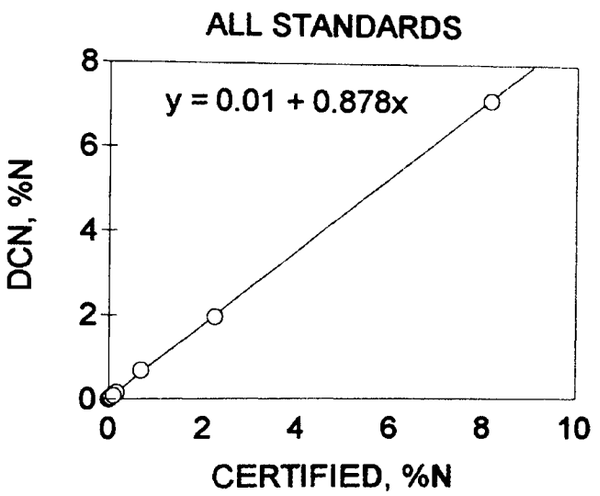
Appendix A. Standard concentrations and percent N observed using dry combustion and micro-Kjeldahl analysis.

STANDARD	CERTIFIED[a] VALUE %N	TKN	DCN
		--------%N--------	
Isothiourea	8.14	8.205±0.001	7.152±0.0178
Apple Leaves	2.24	2.079±0.0314	1.951±0.0094
Corn Stalks	0.689	0.655±0.0166	0.689±0.0272
Phenanthrene	0.178	0.203±0.0065	0.165±0.0036
Tibet Soil*	0.128	0.133±0.0018	0.127±0.0016

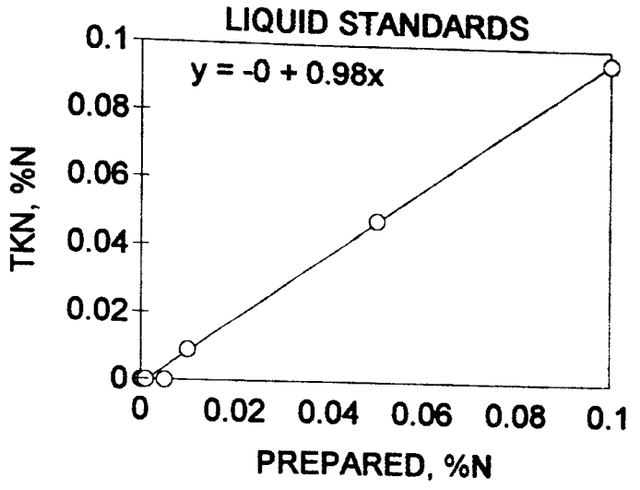
* - used as the reference standard for dry combustion analysis

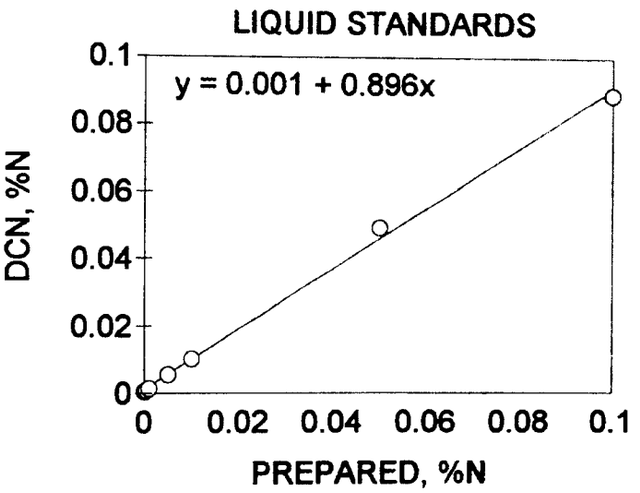
a - apple leaves and corn stalks certified by National Instatute of Standards and Technology Gaithersburg, MD, Tibet soil certified by the Institute of Environmental chemistry Academia Sinica Beijing, China, Isothiourea and Phenanthrene are theoretical values





Appendix B. Linear regression of all standards (certified and prepared liquid) for Kjeldahl N and dry combustion N determinations.





Appendix C. Linear regression of prepared liquid standards for Kjeldahl N and dry combustion N determinations.

Instrument Precision (materials and methods):

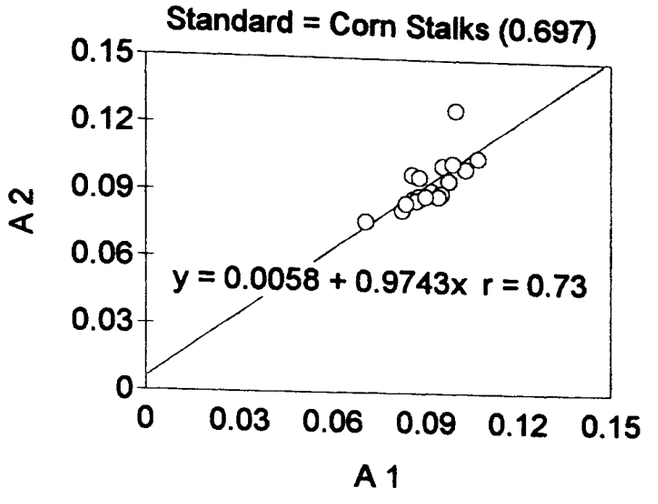
Twenty soils (employing duplicates) were analyzed in duplicate for total N using dry combustion. The analyses were conducted on three separate occasions whereby the instrument was calibrated using corn stalks, phenanthrene and Tibetan soil as the calibration standards. From this analysis, two separate statistical relationships were evaluated; a) precision of duplicate samples using the different calibrations and b) relationship between soil N determined using different calibration standards.

Instrument Precision (results and discussion):

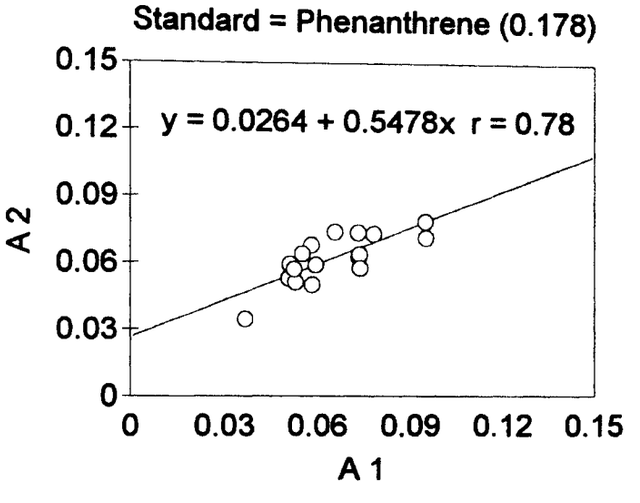
Linear regression of dry combustion soil N analysis (duplicate) using the three certified N standards as instrument calibration materials are illustrated in Appendix E. Simple linear correlation was significantly improved when the Tibetan soil was used as the calibration standard. It was not surprising to observe this response since the Tibetan soil more closely approximated the range at which total N was found in this subsample of local soils (all samples less than the 0.128 % N in the Tibetan soil). The other standards having higher percent N (0.697 and 0.178) essentially widened the analytical range (both X and Y axis). Variability on one axis and not on the other for duplicate samples run using phenanthrene as the calibration standard was not understood.

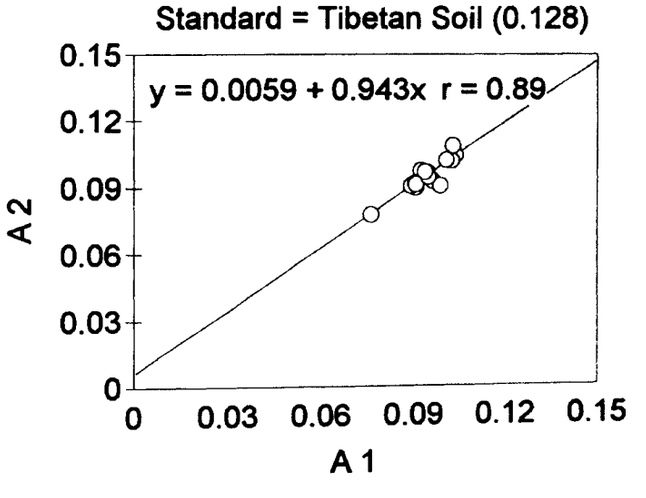
Total N analysis on the same 20 soils using Tibetan soil as the calibration standard and total N using corn stalks as the calibration standard were poorly correlated (Figure 7). The intercept component was significantly different from zero and the slope significantly different from 1.0. When soil N analyzed using phenanthrene as the calibration standard was regressed on soil N analyzed using either the Tibetan soil or corn stalks, correlation was poor.

Because the precision was markedly improved on the duplicate samples using the Tibetan soil as the calibration standard, this work would suggest that analysis for total soil N (range in total N of 0.05% to 0.25%) may be improved by using the Tibetan soil certified standard or using a standard which more closely approximates the range to be analyzed.



A1 = Duplicate #1
A2 = Duplicate #2





Appendix E. Linear regression of duplicate soil samples analyzed using three different certified reference standards.

VITA 2

Michael E. Jojola

Candidate for the Degree of

Master of Science

Thesis: ACCRUACY AND PRECISION OF TOTAL NITROGEN DETERMINATION USING AUTOMATED DRY COMBUSTION

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Professional Experience: Graduate assistant, Department of Agronomy, Oklahoma State University, 1988- 1990;Senior Research Specialist, Soil Fertility, Department of Agronomy, Oklahoma State University, 1990 to present.